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Cytomegalovirus (CMV) Antigenemia Assay Is More Sensitive Than Shell Vial Cultures for Rapid Detection of CMV in Polymorphonuclear Blood Leukocytes

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We compared the cytomegalovirus (CMV) antigenemia assay with shell vial cultures of polymorphonuclear leukocyte (PMNL)-enriched blood fractions for rapid diagnosis of CMV viremia. PMNL fractions of 280 blood specimens from 171 patients (170 solid-organ transplant recipients and 1 patient undergoing pretransplant evaluation) were inoculated in shell vial and conventional CMV cultures. A commercially available kit (CMV-vue kit; INCSTAR Corp.) was used for the CMV antigenemia assay, in which PMNL preparations were stained with monoclonal antibodies directed against the CMV protein pp65. Mixed-leukocyte blood fractions from the same blood specimens were inoculated in parallel shell vial and conventional cultures. CMV viremia (defined by the isolation of CMV in conventional cultures) was detected in 32 (13%) of 245 PMNL fractions included in the final analysis. Twenty-eight (87.5%) were also positive in the CMV antigenemia assay, whereas 22 (69%) were positive in shell vial cultures. Ten (4%) additional PMNL fractions positive only in the CMV antigenemia assay were from eight patients with active CMV infections (six patients), who had previous or subsequent episodes of CMV viremia (seven patients), or in whom CMV was isolated in cultures of simultaneously obtained mixed-leukocyte fractions (three patients). Overall, the CMV antigenemia assay was significantly more sensitive than shell vial cultures for detection of CMV in the PMNL fraction of blood leukocytes ($P < 0.01$, McNemar's test), and we recommend it as the method of choice for rapid diagnosis of CMV viremia.

Cytomegalovirus (CMV) infections are a major cause of morbidity and mortality among immunocompromised individuals (3, 10, 11, 14). Because the diagnosis of CMV infections cannot be made reliably on clinical grounds alone, laboratory confirmation is required. The most specific laboratory method for diagnosis of CMV infection is isolation of the virus in culture. Isolation of CMV from blood leukocytes (CMV viremia) is considered a reliable marker of disseminated CMV infection and predicts invasive CMV disease (12, 16, 17). Therefore, rapid and sensitive methods for detection of CMV viremia are critical to identify patients at risk for developing invasive CMV disease, who may benefit from early antiviral therapy (7). Recently, a new method (CMV antigenemia assay) has been successfully applied to detection of CMV viremia (5, 15, 18, 19, 21). This assay is based on direct detection of the CMV protein pp65 in preparations of polymorphonuclear leukocyte (PMNL) blood fractions (4, 8).

To analyze the potential value of the CMV antigenemia assay as an alternative to shell vial cultures (1, 13) for rapid diagnosis of CMV viremia, we performed a prospective study comparing the CMV antigenemia assay with shell vial cultures of PMNL fractions. However, because mixed-leukocyte (ML) fractions (instead of PMNL fractions) are routinely used in our laboratory for CMV blood cultures, parallel shell vial and conventional cultures of ML fractions obtained from the same blood specimens were also performed.

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MATERIALS AND METHODS

Blood specimens. Blood specimens used in this study were received in the University of Minnesota Diagnostic Virology Laboratory with a request for shell vial and conventional CMV cultures. Only specimens collected in two EDTA-treated tubes with at least 3 ml of blood in each tube (to allow independent separations of PMNL and ML fractions) were evaluated.

CMV antigenemia assay. The CMV antigenemia assay was performed with a commercially available kit (CMV-vue Kit, INCSTAR Corp., Stillwater, Minn.) by following the manufacturer's instructions. PMNL fractions were separated from 3 ml of EDTA-treated blood by sedimentation in a 0.5% dextran solution (INCSTAR Corp.) at 37°C for 10 to 15 min. PMNL were recovered by 10 min of centrifugation at room temperature (300 × *g*) of the PMNL-rich supernatant fraction. Contaminated erythrocytes were lysed in an NH_4Cl solution for 5 min at room temperature. After three washes in phosphate-buffered saline (PBS), PMNL were resuspended in 2 ml of culture medium (minimum essential medium, 2% fetal bovine serum, penicillin G [100 U/ml], gentamicin [50 μg/ml], and amphotericin B [2.5 μg/ml]). PMNL aliquots (25 μl) containing 5×10^4 cells were placed (in duplicate) in wells of microscopy slides (specially coated to facilitate cell adherence). After incubation for 15 min at room temperature, slides were fixed in acetone for 10 min

(two times), air dried, soaked in a solution that inhibits endogenous peroxidase, incubated for 45 min at 37°C in a moist chamber with a mixture of two murine monoclonal antibodies directed against the CMV protein pp65, and stained with a horseradish peroxidase-conjugated anti-mouse immunoglobulin G antibody. Slides were then observed under a light microscope for the presence of red-brown nuclear or perinuclear staining of the CMV-infected cells. Each slide included wells with fibroblasts infected with the CMV strain AD-169 (positive control) and wells with uninfected human diploid fibroblasts (negative control).

Shell vial and conventional cultures of PMNL fractions. Aliquots (0.3 ml) of PMNL suspensions were inoculated (in triplicate) into shell vials containing coverslips covered with monolayers of human diploid fibroblasts. After centrifugation at 700 × *g* for 50 min at room temperature, culture medium (1 ml) was added to each vial. Shell vials were then incubated at 37°C in a 5% CO_2 atmosphere for 16 to 18 h. After removal of the culture medium, shell vials were fixed in methanol at room temperature, stained according to a direct immunofluorescence assay using a murine monoclonal antibody against a 72-kDa immediate-early antigen of CMV (Ortho Cytomegalovirus Identification Reagent; Ortho Diagnostic Systems Inc., Raritan, N.J.) (6), and observed under a fluorescence microscope for the typical apple-green fluorescent nuclear staining of CMV-infected cells.

For conventional cultures, aliquots (0.2 ml) of PMNL suspensions were inoculated (in duplicate) into 24-well tissue culture plates containing confluent monolayers of human diploid fibroblasts. After a 2-h adsorption period, the inoculum was removed and 1 ml of culture medium was added to each well. Plates were incubated at 37°C in a 5% CO_2 atmosphere for 3 weeks, with culture medium changes at 24 h and weekly thereafter. Cultures were observed under a light microscope for the appearance of cytopathic effects characteristic of CMV at 24 h, 72 h, 7 days, and once a week thereafter. When discordant results (i.e., negative conventional cultures with positive shell vial cultures and/or positive CMV antigenemia results) were obtained, cultures were maintained for up to 5 weeks. Days to culture positivity were those elapsed between inoculation of cultures and the first appearance of cytopathic effects.

Shell vial and conventional cultures of ML fractions. ML fractions were obtained from 3 to 5 ml of EDTA-treated blood by gradient centrifugation using Polymorphoprep (Nycomed Pharma AS, Oslo, Norway) solution. Blood samples were layered over 3 ml of Polymorphoprep in a 15-ml conical tube and centrifuged at 650 × *g* for 30 min at room temperature. After centrifugation, all leukocyte bands were transferred to a new tube, washed (three times) in PBS, and resuspended in 2 ml of culture medium. Aliquots of ML suspensions were inoculated in conventional and shell vial cultures, according to the procedures indicated above.

RESULTS

The study was conducted between November 1990 and December 1991 and included 280 blood specimens from 171 patients (170 solid-organ recipients [81 kidney, 35 kidney-pancreas, 21 pancreas, 18 liver, 2 kidney-liver, 11 heart, and 2 heart-lung recipients] and 1 patient undergoing pretransplant evaluation). Thirty-five specimens were not evaluated because of toxicity of the shell vial cultures (18 specimens), malfunction of an incubator that resulted in a short (<3-week) incubation period of conventional cultures (15 specimens), and bacterial or fungal contamination of cultures (2 specimens). Results from 245 blood specimens were included in the final analysis.

TABLE 1. Comparison of shell vial cultures and CMV antigenemia assay with conventional cultures for detection of CMV in PMNL fractions

Result of[a]:		No. of conventional cultures	
CMV antigenemia assay	Shell vial culture	Positive (n = 32)	Negative (n = 213)
+	+	21	5
+	−	7	10
−	+	1	3
−	−	3	195

[a] Sensitivity, specificity, positive predictive values, and negative predictive values were, respectively 87, 92, 65, and 98% (CMV antigenemia assay) and 69, 96, 73, and 93% (shell vial cultures). For the sensitivity values, $P < 0.01$ (McNemar's test).

Comparison of CMV antigenemia assay with shell vial cultures of PMNL fractions. CMV was isolated in conventional cultures from 32 (13%) PMNL fractions. Forty-three (17.5%) PMNL fractions were CMV antigenemia positive, including 21 fractions positive in shell vial and conventional cultures, 5 positive in shell vial cultures, and 10 negative in shell vial and conventional cultures (Table 1). The 10 PMNL fractions positive only in the CMV antigenemia assay were from eight solid-organ transplant recipients, six of whom had CMV diseases, including involvement of the gastrointestinal tract (one patient), lung (one patient), or liver (one patient) or febrile syndromes attributed to CMV (three patients). Six patients were receiving ganciclovir (average length of therapy, 7 days; range, 2 to 15 days). The remaining two patients were asymptomatic when blood samples were drawn. In both cases, CMV was isolated in shell vial and/or conventional cultures of simultaneously obtained ML fractions from the same blood specimens. In seven patients, CMV was isolated in shell vial and/or conventional cultures of ML fractions within 1 month of the CMV antigenemia-positive specimens (Table 2). Thirty (12%) PMNL fractions were positive in shell vial cultures, including 22 fractions positive in conventional cultures and 8 fractions negative in conventional cultures. Three PMNL fractions positive in shell vial cultures but negative in conventional cultures and in the CMV antigenemia assay were from three patients (two with episodes of organ rejection and one who was asymptomatic); CMV was isolated in conventional cultures of simultaneously obtained ML fractions in two patients. Sensitivity, specificity, and positive and negative predictive values of the CMV antigenemia assay and shell vial cultures are shown in Table 1, footnote *a*. The CMV antigenemia assay was significantly more sensitive than shell vial cultures for detection of CMV viremia ($P < 0.01$, McNemar's test), whereas positive and negative predictive values of both assays were not statistically different.

Comparison of PMNL and ML fractions for detection of CMV in culture. Detection of CMV in conventional and shell vial cultures of PMNL fractions was compared with that in cultures of ML fractions. Of 43 blood specimens positive by any culture method, 40 (93%) were positive in conventional cultures, including 37 (92.5%) cultures of ML fractions and 32 (80%) cultures of PMNL fractions. Days to isolation of CMV were 14 ± 7 days (range, 6 to 35 days) for cultures of ML fractions and 13.8 ± 6.3 days (range, 6 to 29 days) for cultures of PMNL fractions. Thirty-four (79%) of the positive blood specimens were detected in shell vial cultures,

TABLE 2. Summary of clinical and laboratory data from patients with PMNL fractions positive only in the CMV antigenemia assay

Patient	No. of specimens	Transplant type	CMV disease	Therapy[a]	Day(s)[b] when CMV was isolated from ML fraction
A	2	Kidney	Gastrointestinal	GCV (12, 15)	−2, +22
B	1	Kidney	None	No	−33, −31, −21, 0, +22
C	1	Kidney	None	No	0
D	2	Kidney	Fever	GCV (2, 10)	−9, 9
E	1	Kidney-pancreas	Fever	GCV (2)	−10, 0
F	1	Pancreas	Pneumonitis	GCV (9)	−8
G	1	Liver	Hepatitis	GCV (3)	−8, −6, −4, −2
H	1	Liver	Fever	GCV (3)	−3

[a] GCV, ganciclovir. Numbers in parenthesis are days of GCV therapy when CMV antigenemia-positive fractions were drawn.
[b] Day 0 is the day when CMV antigenemia-positive fractions were obtained.

including 29 (67%) shell vial cultures of ML fractions and 28 (65%) shell vial cultures of PMNL fractions.

DISCUSSION

This study demonstrated that the CMV antigenemia assay is more sensitive than shell vial cultures of PMNL fractions for rapid diagnosis of CMV viremia (defined by the isolation of CMV in conventional cultures). With this assay, CMV was detected in 87.5% (28 of 32) of the PMNL fractions from which CMV was isolated in conventional cultures, whereas shell vial cultures identified 69% (22 of 32) of the culture-positive PMNL fractions. We also identified 10 (4%) PMNL fractions that were positive only in the CMV antigenemia assay. Most likely, these were not false-positive results of the CMV antigenemia assay, as evidenced by the isolation of CMV in cultures of ML fractions simultaneously obtained (three patients), preceding and/or subsequent episodes of CMV viremia (seven patients), and evidence of CMV disease (six patients). Discrepant results between the CMV antigenemia assay and CMV cultures have been previously reported and appear to be the consequence of antiviral therapy or sample variability (5). In a study by Gerna et al., 21 (10%) of 200 culture-negative blood samples (obtained from 14 heart transplant patients over a 2- to 3-month period after transplantation) were positive in the CMV antigenemia assay (5). Twelve (57%) of the CMV antigenemia-positive fractions were from patients treated with ganciclovir. In our study, 8 of the 10 blood samples positive only in the CMV antigenemia assay were from patients receiving ganciclovir when blood samples were drawn. This dissociation between CMV antigenemia and culture results observed during ganciclovir therapy could possibly be due to a ganciclovir-induced block in viral DNA replication (and consequent inhibition of infection of fibroblasts in cultures) combined with intact expression of CMV antigens in infected PMNL (5, 19). On the basis of these observations, some investigators have recommended that the CMV antigenemia assay should be used for monitoring patients during ganciclovir therapy (5).

In previous studies, the CMV antigenemia assay was performed according to the technique initially described by van der Bij et al. (22), in which cytospin preparations of dextran-separated PMNL fractions were stained with monoclonal antibodies directed against the pp65 protein of CMV. One technical advantage of our study is that cytospin preparations were not used. Instead, suspensions of PMNL were spotted onto specially coated slides (included in the kit). The slides were also treated to inhibit endogenous peroxidase activity of blood leukocytes (with a solution included in the kit). In our experience, background staining of endogenous peroxidase did not interfere with reading of the slides.

Our study permitted us to directly compare PMNL fractions with ML fractions for detection of CMV in shell vial and conventional cultures. CMV was isolated more often in conventional cultures of ML fractions than in cultures of PMNL fractions (37 versus 32 positive cultures). There were no differences between ML and PMNL fractions with respect to days to isolation of CMV in conventional cultures (14 ± 7 versus 14 ± 6 days) or results of shell vial cultures (29 versus 28 positive cultures). The higher isolation rate of CMV from ML fractions is consistent with an earlier report that the isolation rate of CMV was higher from combined mononuclear and polymorphonuclear fractions than from the separate fractions (9) and recent molecular studies demonstrating that monocytes are an important site of infection during CMV viremia (2, 17, 20). Because of the higher detection of CMV in ML fractions, a study evaluating the CMV antigenemia assay using ML preparations is currently under way in our laboratory.

What are the implications of our findings for the diagnostic virology laboratory? Several authors have advocated using a combination of methods (CMV antigenemia assay, shell vial cultures, and conventional cultures) to achieve maximum sensitivity for detection of CMV viremia (5, 19, 21). However, this approach is impractical and expensive. On the basis of our results, we recommend the CMV antigenemia assay as the preferred method for rapid diagnosis of CMV viremia. Because not all viremias are detected with this assay, results should be verified with conventional cultures.

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